

Listing of Claims:

1. (Currently Amended) An α_2 -antiplasmin cleaving enzyme, comprising:

a purified protein having a molecular weight of about 180 kDa in a dimeric form as determined by SDS-PAGE, wherein each subunit of the dimeric form has a molecular weight of about 97 kDa as determined by SDS-PAGE, and has an N-terminal sequence having an N-terminal amino acid and wherein the protein comprising an N-terminal sequence comprising is SEQ ID NO:1 and the N-terminal amino acid is isoleucine, and the protein further comprising internal sequences comprising SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8, and wherein the enzyme cleaves precursor α_2 -antiplasmin at the pro12-asn13 bond.

2. (Original) The α_2 -antiplasmin cleaving enzyme of claim 1 having an optimum activity at pH=7.5 \pm 0.2.

3. (Withdrawn) A method of screening for inhibitors of antiplasmin cleaving enzyme, comprising:

providing a fluorescent resonance energy transfer peptide comprising a P₁-P₁' bond and comprising a fluorophore and a quenching group separated by the P₁-P₁' bond wherein the P₁ comprises a proline or proline analog and the P₁' comprises an asn, ser, tyr, or other amino acid such that the peptide can be cleaved by α_2 -antiplasmin cleaving enzyme;

providing a quantity of α_2 -antiplasmin cleaving enzyme;

exposing the α_2 -antiplasmin cleaving enzyme to an α_2 -antiplasmin cleaving enzyme inhibitor candidate to form a test mixture;
combining the test mixture with the fluorescent resonance energy transfer peptide; and
measuring the fluorescence emission from the test mixture to identify whether or not the α_2 -antiplasmin cleaving enzyme inhibitor candidate inhibits the activity of α_2 -antiplasmin cleaving enzyme.

4. (Withdrawn) The method of claim 3 wherein the fluorescent resonance energy transfer peptide comprises the quenching group upstream of the proline-asparagine bond and the fluorophore downstream of the proline-asparagine bond.

5. (Withdrawn) The method of claim 3 wherein the fluorescent resonance energy transfer peptide comprises the quenching group downstream and the fluorophore upstream of the proline-asparagine bond.

6. (Withdrawn) The method of claim 3 wherein the P_1 - P_1' bond is a proline-asparagine bond.

7. (Withdrawn) The method of claim 3 wherein the fluorescent resonance energy transfer peptide comprises SEQ ID NO:9.

8. (Withdrawn) The method of claim 3 wherein the α_2 -antiplasmin cleaving enzyme comprises:

a protein having a molecular weight of about 180 kDa in a dimeric form

as determined by SDS PAGE, wherein each subunit of the dimeric form has a molecular weight of about 97 kDa as determined by SDS PAGE, the protein comprising an N-terminal sequence comprising SEQ ID NO:1 and internal sequences comprising SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8, and wherein the enzyme cleaves precursor α_2 -antiplasmin at the pro 12-asn 13 bond.

9. (Withdrawn) An inhibitor of α_2 -antiplasmin cleaving enzyme identified by the screening method of claim 3.

10. (Withdrawn) An inhibitor of antiplasmin cleaving enzyme which is effective in binding to or blocking the α_2 -antiplasmin binding site, or α_2 -antiplasmin pro-asn or cleaving site of antiplasmin cleaving enzyme.

11. (Withdrawn) A method for identifying an enzyme inhibitor, comprising:

combining a dimeric molecule having α_2 -antiplasmin cleaving enzymatic activity, said dimeric molecule having a molecular weight of about 180 kilodaltons as determined by SDS-PAGE, a substrate for said molecule, and a test substance to be tested as an enzyme inhibitor; and

determining activity of the dimeric molecule on the substrate, wherein a decrease in activity of the dimeric molecule when the test substance is present indicates that said test substance is an inhibitor.

12. (Withdrawn) A monoclonal antibody raised against α_2 -antiplasmin cleaving enzyme which binds to an α_2 -antiplasmin binding portion of the α_2 -antiplasmin cleaving enzyme.

13. (Withdrawn) The monoclonal antibody of claim 12 wherein the monoclonal antibody binds to an active site of α_2 -antiplasmin cleaving enzyme which cleaves the pro-asn bond of precursor α_2 -antiplasmin.

14. (Withdrawn) A method of screening a subject for risk for atherosclerosis or complications of atherosclerosis or for diseases related to fibrin deposition, comprising:

determining the identity of the amino acid at position 6 in α_2 -antiplasmin of the subject.

15. (Withdrawn) The method of claim 14 wherein in the step of determining the identity of the amino acid at position 6, when the amino acid is tryptophan, the subject is diagnosed as having a reduced risk for atherosclerosis or complications of atherosclerosis or for diseases related to fibrin deposition.

16. (Withdrawn) The method of claim 14 wherein in the step of determining the identity of the amino acid at position 6, when the amino acid is arginine, the subject is diagnosed as having an enhanced risk for atherosclerosis or complications of atherosclerosis or for diseases related to fibrin deposition.

17. (Withdrawn) The method of claim 14 comprising the step of obtaining a biological sample from the subject and using a PCR reaction to analyze the biological sample for a trp6arg polymorphism in a portion of the α_2 -antiplasmin gene.

18. (Withdrawn) The method of claim 17 wherein the PCR reaction uses a forward primer comprising SEQ ID NO:10 and a reverse primer comprising SEQ ID NO:11.

19. (Withdrawn) A method of inhibiting digestion by plasmin in a subject in need of such therapy, comprising:

administering to the subject a therapeutically effective quantity of α_2 -antiplasmin cleaving enzyme effective in enhancing the availability of soluble activated α_2 -antiplasmin in the subject's plasma, the α_2 -antiplasmin cleaving enzyme comprising a protein having a molecular weight of about 180 kDa in a dimeric form as determined by SDS PAGE, wherein each subunit of the dimeric form has a molecular weight of about 97 kDa as determined by SDS PAGE, the protein comprising an N-terminal sequence comprising SEQ ID NO:1 and internal sequences comprising SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8, and wherein the enzyme cleaves precursor α_2 -antiplasmin at the pro12-asn13 bond.

20. (Withdrawn) A method of producing activated α_2 -antiplasmin, in vitro, comprising:

combining a quantity of precursor α_2 -antiplasmin with a quantity of α_2 -antiplasmin cleaving enzyme under conditions suitable for cleavage of the precursor α_2 -antiplasmin by the α_2 -antiplasmin cleaving enzyme, the α_2 -antiplasmin cleaving enzyme comprising:

a protein having a molecular weight of about 180kDa in a dimeric form as determined by SDS PAGE, wherein each subunit of the dimeric form has a molecular weight of about 97 kDa as determined by SDS PAGE, the protein comprising an N-terminal sequence comprising SEQ ID NO:1 and internal sequences comprising SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8, and wherein the enzyme cleaves precursor α_2 -antiplasmin at the pro12-asn13 bond.

21. (Withdrawn) A method of enhancing fibrin digestion in vivo, comprising providing to a subject in need of clot digestion or clot prevention, simultaneously or in sequence, a quantity of plasminogen activator and an inhibitor of antiplasmin cleaving enzyme, wherein the quantity of plasminogen activator is less than the amount provided in standard therapeutic protocol absent the inhibitor of antiplasmin cleaving enzyme.